Exhibit 4

Journal of Medicinal Chemistry

Volume 29, Number 7 July 1986

1329

Perspective

Drug Development for Senile Cognitive Decline

Fred M. Hershenson* and Walter H. Moos

Department of Chemistry, Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan 48105.
Received February 17, 1986

Introduction. The treatment of senile[1] cognitive decline is one of the greatest challenges in the health sciences today. No truly effective therapy has yet been launched; thus research in the cognitive sciences has the potential to produce enormous medical benefits. For the many scientists working to find a cognition activator with robust effects, the risk lies in the possibility that senile cognitive decline may not be treatable. In this paper, we hope to bring relevant data on senile cognitive decline into a meaningful relationship, thus providing a functional perspective for further research. Readers are reminded that this is a Perspective, not a Review. More comprehensive accounts can be found in the recent literature.[2,3]

Dementia is a clinical syndrome involving reduced intellectual functioning with impairments in memory, language, visuospatial skills, and cognition (including mathematics, abstraction, and judgment).[4] Currently, several dementias can be treated (Table I), but others cannot, most notably primary degenerative dementia (PDD; also called senile dementia, senile dementia of the Alzheimer type, Alzheimer disease, organic brain syndrome).

Many health problems contribute to senile cognitive decline, including PDD, mild (or minimal) memory impairment (also called benign senescent forgetfulness), and multiinfarct dementia. The most common accepted form of senile cognitive decline is PDD. *While better drugs are still needed for treatable dementias, untreatable cognitive disorders, particularly PDD, present the greatest challenges and will be the focus of this Perspective.*

Table I. Treatable Dementias[a]

intracranial conditions	
	multiinfarct dementia (MID)
	extrapyramidal disorders (EPS)
	hydrocephalus
	subdural hematomas
	intracranial neoplasms
	infections
chemical intoxications	
	drugs
	metals
	industrial waste
depression	
systemic disorders	
	cardiovascular
	hepatic
	endocrine
	renal
	nutritional deficiencies
	collagen-vascular diseases

The original diagnosis of PDD was made in 1907 by Alois Alzheimer.[5] Alzheimer reported on a 56-year-old woman who had died following a 5–6-year illness characterized by personality changes, disorientation, and memory loss. Postmortem microscopic examination of brain tissue taken from this patient revealed high densities of lesions that are currently described as neuritic plaques and neurofibrillatory tangles. The microscopic changes had previously been observed only in the brains of people over 70 years of age; however, the relationship between normal aging of the brain and PDD remains unresolved.[7]

PDD was considered a medical curiosity for many years; however, the magnitude of its occurrence, especially in the elderly, has only been appreciated within the past decade. Data from population studies suggest a 10- to 20-fold in-

(1) The term "senile", per se, refers only to old age, not to a mental disorder. We will use the phrase "senile cognitive decline" to denote the variety of cognitive disorders observed in the elderly.

(2) See, for example, Bonby, J.; Bonalli, A.; Vergna, L.; Stirne, J.; Carnanese, G. *J. Am. Geriatr. Soc.* **1985**, *33*, 266. Blass, J. P. *Disease-a-Month* **1985**, *31*, 1. Hutton, J. T.; Kenny, A. D., Eds. *Senile Dementia of the Alzheimer Type*; Alan R. Liss: New York, 1986.

(3) A particularly good collection of articles on Alzheimer disease and related disorders can be found in Roth, M.; Iversen, L. L., Eds. *Br. Med. Bull.* **1986**, *42* (1).

(4) Cummings, J.; Benson, D. F.; LoVerme, S., Jr. *J. Am. Med. Assoc.* **1980**, *243*, 2434. *Diagnostic and Statistical Manual of Mental Disorders*, 3rd ed.; American Psychiatric Association: Washington, DC, 1980 [commonly referred to as DSM-III]. For suggested improvements to DSM-III, see, for example, Jorm, A. F.; Henderson, A. S. *Br. J. Psychiatry* **1986**, *147*, 594.

(5) Cummings, J. L. *Clin. Ther.* **1985**, *7*, 490.

(6) For a translation of the original report, see Wilkins, R. H.; Brody, I. A. *Arch. Neurol.* **1969**, *21*, 109.

(7) A review of the biochemical characteristics of PDD is beyond the scope of this article. For reviews, see, for example, Thienhaus, O. J.; Hartford, J. T.; Skelly, M. F.; Bozmann, H. B. *J. Am. Geriatr. Soc.* **1985**, *33*, 715. Gottfries, C. G. *Psychopharmacology* **1985**, *86*, 245. Rathbaun, K. L.; Conner, C. R. *Drug Install. Clin. Pharm.* **1984**, *18*, 834. Price, D. L.; Kitt, C. A.; Struble, R. G.; Whitehouse, P. J.; Cork, L. C.; Walker, L. C. *Ann. N.Y. Acad. Sci.* **1985**, *457*, 36.

0022-2623/86/1829-1129$01.50/0

crease in the prevalence of PDD between ages 80 and 80, and the incidence of PDD will increase in the coming years as the geriatric segment of the population grows. In the United States alone, the segment of the population presently over 65 is estimated at 11% or 25 million people. Over the next 50 years this figure should grow to 55 million or 20% of the population.[8]

The scientific study of PDD has been hampered by (1) the lack of an early, reliable diagnostic method, (2) an unknown etiology, (3) little knowledge about the homogeneity or heterogeneity of the disease,[9] and (4) the absence of effective therapeutic agents and appropriate animal models.

The onset of PDD is insidious, usually taking several years before either the affected individual or close family members recognize that a medical problem may exist. The earliest symptom is forgetfulness (e.g., recent events, names of individuals, locations of objects). While the patient manages daily activities during the early phase of PDD, routine tasks become increasingly difficult as the disease progresses. The patient becomes disoriented, confused, and experiences emotional changes, most frequently those of depression. Occasionally, hallucinations accompany the behavioral changes. In the final stages of PDD, neurological functions fail, and the ability to move and communicate is eventually lost. A Global Deterioration Scale has been developed to categorize the severity of the disease based on behavioral characteristics.[10] PDD is most frequently observed in individuals over age 50, and while the progression of the disease is somewhat variable, it is usually faster when the onset occurs at an earlier age.

Diagnosis. Primary degenerative dementia is currently diagnosed by excluding other possible causes of the observed behavioral manifestations. Neuropsychological tests, including the mini-mental status questionnaire[11] and the behavioral test of Blessed[12] are used to assess the degree of dementia. Other possible causes, including those mentioned above (Table I), are excluded on the basis of clinical history or laboratory data. For example, multiinfarct dementia, the second most common form of dementia, is excluded by using Hachinski criteria.[13] and laboratory examination of blood and urine samples is used to rule out factors such as vitamin B_{12} deficiency or drug intoxication.

Unfortunately, no objective, unequivocal diagnostic procedure is presently available for early detection of PDD or quantification of cognitive decline. New imaging techniques such as positron emission tomography[14] and magnetic resonance may provide insights into differences in brain functioning between PDD patients and age-matched controls; however, these methods are not yet suited for evaluating large numbers of patients routinely. Other laboratory measures involving multichannel computer-analyzed electroencephalography (EEG), cerebral blood flow monitoring,[15] computerized tomography of brain mass, and analysis of cerebrospinal fluid may provide useful markers that are more easily obtained and quantified. PDD patients may display greater sensitivity to certain pharmacological agents (e.g., the anticholinergic scopolamine) than normals, thus allowing a more accurate assessment of their disorder.[16] Evoked potential recording may be of value in diagnosing early PDD.[17] Other differences may eventually be exploited (e.g., fingerprint patterns,[18] hyperammonemia[19]); however, much research must be done before such methods can be established as valid diagnostic tools. Success in developing rapid and reliable diagnostic procedures will ultimately play an important role in the clinical development of new therapeutic agents.

Table II. Possible Causes of PDD[20]

genetic factors
abnormal protein models
infectious agents
toxins
blood flow disorders
cholinergic hypothesis
multiple factors

Etiology. The etiology and pathogenesis of PDD is presently unclear; however, a number of factors have been hypothesized to be involved (see Table II). Questions exist whether PDD is a single entity or two disorders; one with an onset before age 65 (presenile dementia), and a second with symptoms appearing in later life (senile dementia). This issue has not been resolved.

The possibility that PDD can be inherited has been a subject of interest for some time. Results from several studies suggest a genetic predisposition to PDD, especially in cases of early onset.[21] Close relatives of PDD patients have a fourfold greater chance of developing the disease than the general population.[22]

Recently, the possibility that chromosomal abnormalities may be involved in the etiology of the disease has been proposed because many individuals with Down's syndrome who reach age 40 develop Alzheimer-type brain lesions and clinical dementia.[23] Additionally, PDD and Down's syndrome share a unique cerebrovascular amyloid fibril protein.[24]

Evidence suggesting that PDD is an infectious disease, possibly of viral origin, is based on certain clinical and neuropathological similarities between PDD and Creutz-

(6) Goodwick, P.; Gershon, S. *J. Clin. Psychiatry* **1984**, *45*, 196.
(9) Mayeux, R.; Stern, Y.; Spanton, S. *Neurology* **1985**, *35*, 453.
(10) Reisberg, B.; Ferris, S. H.; DeLeon, M. J.; Crook, T. *Am. J. Psychiatry* **1982**, *139*, 1136.
(11) Folstein, M.; Folstein, S.; McHugh, P. R. *J. Psychiatr. Res.* **1975**, *12*, 189.
(12) Blessed, G.; Tomlinson, B. E.; Roth, M. *Br. J. Psychiatry* **1968**, *114*, 797.
(13) Rosen, W. G.; Terry, R. D.; Fuld, P.; Katzman, R.; Peck, A. *Ann. Neurol.* **1980**, *7*, 486.
(14) Ferris, S. H.; DeLeon, M. J.; Wolf, A. P.; George, A. E.; Reisberg, B.; Christman, D. R.; Yoshiura, Y.; Fowler, J. S. *Adv. Neurol.* **1983**, *38*, 123. Friedland, R. P.; Budinger, T. F.; Brant-Zawadzki, M.; Jagust, W. J. *J. Am. Med. Assoc.* **1984**, *252*, 2750. See also Holman, B. L.; Gibson, R. E.; Hill, T. C.; Eckelman, W. C.; Albert, M.; Reba, R. C. *J. Am. Med. Assoc.* **1985**, *254*, 3063.
(15) Hagberg, B.; Ingvar, D. H. *Br. J. Psychiatry* **1976**, *128*, 208.
(16) Sunderland, T.; Tariot, P.; Murphy, D. L.; Weingartner, H.; Mueller, E. A.; Cohen, R. M. *Psychopharmacology* **1985**, *87*, 247.
(17) St. Clair, D. M.; Blackwood, D. H. R.; Christie, J. E. *Br. J. Psychiatry* **1985**, *147*, 702.
(18) Wolozin, H. J. *Arch. Neurol.* **1985**, *42*, 80.
(19) Fleman, M.; Gordon, E.; Filaki, Y.; Halmes, R.; Appell, J.; Rabbani, K. *Am. J. Psychiatry* **1985**, *142*, 71.
(20) Wortman, R. J. *Sci. Am.* **1985**, *252*, 62. Ferry, G. *New Scientist* **1985**, 33.
(21) Heston, L. L.; Mastri, A. R.; Anderson, V. E.; White, J. *Arch. Gen. Psychiatry* **1981**, *38*, 1085.
(22) Glenner, G. G. *J. Am. Geriatr. Soc.* **1981**, *30*, 59.
(23) Price, D. L.; Whitehouse, P. J.; Stribble, R. G. *Ann. N.Y. Acad. Sci.* **1982**, *396*, 145. Tanzi, R. E.; Heston, L. L.; Davies, P.; Hardy, J. V.; Schapiro, M. B. *Ann. Internal Med.* **1985**, *103*, 586. Mortimer, C. J.; Rissing, G.; Weinman, N. A.; Multhaup, G.; McDonald, B. L.; Beyreuther, K. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4245.
(24) Glenner, G. G.; Wong, C. W. *Biochem. Biophys. Res. Commun.* **1984**, *122*, 1131.

feldt–Jakob disease (CJD). CJD is a rare disorder of progressive dementia accompanied by movement disturbances that is followed by death within 1–2 years from onset. The infectious agent may be a slow virus because an incubation period of several years is required between exposure to the agent and the first symptoms. Scrapie, a brain disorder of sheep and goats, is an infectious disease that may also involve slow viruses. Both can be transmitted by injecting extracts of infected brain tissue.

Prusiner and co-workers have recently demonstrated the infectious pathogen in scrapie to be a protein particle termed a prion.[25] Prions are defined as small, proteinaceous, infectious particles that resist inactivation by procedures that modify nucleic acids. All attempts to demonstrate the existence of nucleic acids within the scrapie agent have failed—how such proteins replicate without genetic material has not been satisfactorily answered.

The rodlike structures observed upon microscopic examination of sheep brains infected with scrapie are thought to be prion aggregates, but these aggregates are not the same as the neuritic plaques seen in PDD.

The transmission of PDD from human brain tissue to experimental animals has not been successful. Establishment of suitable animal models reflecting an infectious type of PDD may be confounded by excessively long incubation periods that exceed the animal's normal life span.

If an infectious agent like a slow virus or a scrapie-like prion is involved in PDD, other factors may be required before the disease can be fully manifested. These may include a genetic predisposition, as mentioned above, or exposure to environmental toxins. Changes in the blood–brain barrier may occur in PDD, thereby causing an increased permeability of the microvasculature that contributes to the observed pathology.[26]

Neurochemical analysis of neuritic plaques is another area of active research. Whether plaques are end products of the pathological process or simply contributors to the disease is not known. Nevertheless, an understanding of the chemical nature of these morphological markers may provide direction in designing new therapeutic agents. Cholinergic, catecholaminergic, and somatostatinergic processes are present in plaques along with proteinaceous material (amyloid).[27] Amyloid is also found in cerebral blood vessels, and leakage of amyloid from vessels into brain tissue has been postulated to trigger the neurotoxicity observed in PDD.[23] Amyloid may originate from a blood-borne precursor protein, being formed in cerebral blood vessels by action of a local enzyme.

The presence of elevated aluminum levels in the brain tissue of PDD patients was originally used to suggest this metal as a causative factor in the disease.[28] While comparisons of brain aluminum levels in PDD patients vs. age-matched controls show little difference,[29] an inorganic substance composed of aluminum and silicon is present in the plaques found in PDD.[28] This remains a controversial area because patients suffering from aluminum toxicity do not exhibit the neuropathological changes characteristic of PDD.[30]

Recent studies involving nerve growth factors suggest a possible new direction for research on the etiology of senile cognitive decline, but more work is needed.[31]

Finally, the function of the immune system[32] in the pathogenesis of PDD is under intense study, but conclusions at this time would be premature. For example, conflicting reports[33,34] have appeared regarding the correlation of levels of serum immunoglobulins A and G with the degree of cognitive impairment in PDD. A genetic factor may be responsible for changes in the immune system of PDD patients.

Past Strategies. The drugs currently used in the treatment of PDD are of questionable value. The earliest therapeutic strategies used agents that improve cerebral blood flow or are mild psychostimulants. In the United States, dihydroergotoxine, the vasodilators papaverine, isoxsuprine, and cyclandelate, and the stimulants methylphenidate and pentylenetetrazole, have been approved for the treatment of senile cognitive decline.[35] Dihydroergotoxine, a mixture of three dihydrogenated ergot alkaloids, is the most widely used drug of this group. *None of these agents has been demonstrated to improve cognition unequivocally in PDD patients.*

Compounds that improve cerebral blood flow through vascular mechanisms have been employed in some countries to treat PDD. These compounds include naftidofuryl, pentoxifylline, suloctodil, vincamine, and calcium channel blockers (e.g., nimodipine). The use of these agents is debatable since a vascular origin for PDD is no longer widely accepted.

A group of agents termed nootropics have been developed on the basis of the observation that the pyrrolidone piracetam facilitates learning and memory in animal models. Human studies with piracetam continue to give conflicting results. Several compounds appear to be more potent than piracetam and have been evaluated clinically in patients with cognitive decline (see Table III).[36] Initial reports from open-label studies have often been encouraging, but well-designed, double-blind, placebo-controlled trials have thus far failed to confirm clear-cut drug effects.

Present Strategies. The focus of research has now shifted to biochemical and neurochemical approaches, with the hope of identifying agents that improve the behavioral endpoints of learning and memory by a defined mechanism of action. Present strategies include cholinergic agents

Table III. Representative Nootropics

piracetam
oxiracetam
pramiracetam (CI-879)
rolziracetam (CI-911)
aniracetam
CI-933
CI-844

(28) Prusiner, S. B.; McKinley, M. P.; Groth, D. P.; Bowman, K. A.; Mock, N. I.; Cochran, S. P.; Masters, F. R. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 6675. Prusiner, S. B. *Science (Washington, D.C.)* **1982**, *216*, 136.

(29) Wisniewski, H. M.; Kozlowski, P. B. *Ann. N.Y. Acad. Sci.* **1982**, *396*, 119–129.

(30) Perry, E. K.; Perry, R. H. *Trends Neurosci.* **1983**, *6*, 201.

(31) Crapper, D.; Kirshen, S. R.; Dalton, A. J. *Science (Washington, D.C.)* **1973**, *180*, 511. King, R. G. *Med. Hypotheses* **1984**, *14*, 301.

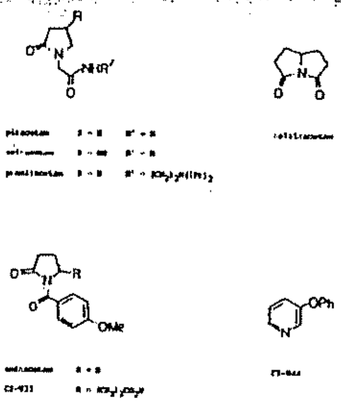
(32) McDonough, W.; Smith, A. L.; Iqbal, K.; Wisniewski, H. M. *Neurology* **1979**, *29*, 809.

(33) Cooke, D.; Elsdorfer, C. *Br. J. Psychiatry* **1968**, *136*, 83.

(34) Miller, A. E.; Neighbour, P. A.; Kirkland, R.; Thomson, R.; Lipkowitz, R. *Ann. Neurol.* **1981**, *10*, 106.

(35) Crook, T. *Ann. N.Y. Acad. Sci.* **1986**, *444*, 415.

(36) See references cited in *Drugs Future* **1985**, *10*, 972, 988.



(e.g., arecoline,[37,38] physostigmine,[38] RS-86,[39] bethanecol,[40] BM-5[41]), analogues of ACTH (e.g., ORG 2766[42]), vasopressin (e.g., DDAVP[42], DGAVP[44]), and somatostatin (e.g., L-363,586[43]), serotonin agents (e.g., alaproclate[46], zimelidine[47]), and adrenergic agents (e.g., clonidine[48]). The most

Table IV. Agents That May Enhance Muscarinic Neurotransmission in Diseases Characterized by a Muscarinic Cholinergic Deficiency[a]

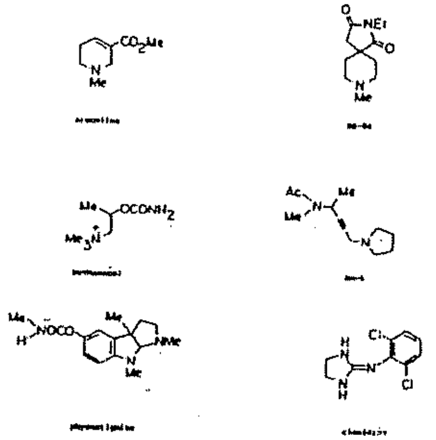
class	example[b]
presynaptic muscarinic antagonist	scopolamine
presynaptic allosteric muscarinic inhibitor	gallamine
presynaptic enhancer of acetylcholine release	aminopyridines
enhancer of high affinity choline uptake	?
reversible inhibitor of acetylcholinesterase	physostigmine
postsynaptic muscarinic agonist	arecoline, oxotremorine
postsynaptic allosteric muscarinic activator	?

[a] None of these appear to be selective for pre- or postsynaptic sites. However, see ref 41 (BM-5).

Table V. Correlation between Electroencephalography and Behavior

EEG band	behavior
alpha (8-12 Hz)	attentional demands
beta (16-24 Hz)	emotion, cognition
theta (2-7 Hz)	cognition (particularly hippocampal theta)

widely accepted biochemical hypothesis, at present, involves the cholinergic system, which is discussed in more detail below.



Biological Models. In order to develop new therapeutic agents in a rational and efficient manner, satisfactory biological models are needed. *Unfortunately, appropriate animal models do not yet exist.* Many considerations are important in developing effective animal models. For example, the animal model should be sensitive and selective for certain types of memory, and confirmation that memory is required in normal animals for accurate performance is essential. The performance of animals with altered brain function should be comparable to similar modulation of human memory. Finally, nonmemory psychological processes must be excluded as possible causes of behavioral changes.

The validity of animal models of cognition is ultimately tested by their ability to predict or at least explain brain mechanisms involved in normal memory, pathological changes that produce memory impairments, and there-

(37) Flood, J. F.; Smith, G. E.; Cherkin, A. *Psychopharmacology* 1985, *86*, 61. Mutschler, E.; Lambrecht, G. *Trends Pharmacol. Sci.* 1984, Suppl., 39.

(38) Mohs, R. C.; Davis, B. M.; Johns, C. A.; Mathe, A. A.; Greenwald, B. S.; Horvath, T. B.; Davis, K. L. *Am. J. Psychiatry* 1985, *142*, 28. Christie, J. E.; Shering, A.; Ferguson, J.; Glen, A. I. M. *Br. J. Psychiatry* 1981, *138*, 46. Brinkman, S. D.; Gershon, S. *Neurobiol. Aging* 1983, *4*, 139. Jotkowitz, S. *Ann. Neurol.* 1983, *14*, 690. Davis, K. L.; Mohs, R. C. *Am. J. Psychiatry* 1982, *139*, 1421. Davis, K. L.; Mohs, R. C.; Tinklenberg, J. R.; Pfefferbaum, A.; Hollister, L. E.; Kopell, B. S. *Science (Washington, D.C.)* 1978, *201*, 272. Smith, C. M.; Swash, M. *Lancet* 1979, 42. Beller, S. A.; Overall, J. E.; Swann, A. C. *Psychopharmacology* 1985, *87*, 147.

(39) Wettstein, A.; Spiegel, R. *Psychopharmacology* 1984, *84*, 572.

(40) Harbaugh, R. E.; Roberts, D. W.; Coombs, D. W.; Saunders, R. L.; Reeder, T. M. *Neurosurgery* 1984, *15*, 514.

(41) Nordstrom, O.; Alberts, P.; Westlind, A.; Unden, A.; Bartfai, T. *Mol. Pharmacol.* 1983, *24*, 1.

(42) Nicholson, A. N.; Stone, B. M.; Jones, S. J. *Eur. J. Clin. Pharmacol.* 1984, *27*, 561. Jolkkonen, J. T.; Soininen, H. S.; Riekkinen, P. J. *Life Sci.* 1985, *37*, 585. Nyakas, C.; Veldhuis, H. D.; DeWied, D. *Brain Res. Bull.* 1985, *15*, 257.

(43) Beckwith, B. E.; Till, R. E.; Schneider, V. *Peptides* 1984, *5*, 819. Stein, D.; Bannet, J.; Averbuch, I.; Landa, L.; Chazan, S.; Belmaker, R. H. *Psychopharmacology* 1984, *84*, 566.

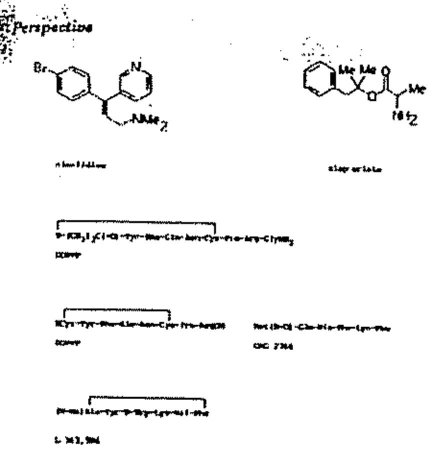
(44) Peabody, C. A.; Thiemann, S.; Pigache, R.; Miller, T. P.; Berger, P. A.; Yesavage, J.; Tinklenberg, J. R. *Neurobiol. Aging* 1985, *6*, 95. Jennekens-Schinkel, A.; Wintzen, A. R.; Lanser, J. B. K. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 1985, *9*, 273.

(45) Cutler, N. R.; Haxby, J. V.; Narang, P. K.; May, C.; Burg, C.; Reines, S. A. *N. Engl. J. Med.* 1985, *312*, 725.

(46) Dahlin, O.; Hedenrud, B.; Jansson, P.; Norgard, J. *Acta Psychiatr. Scand.* 1985, *71*, 190. Bergman, I.; Brane, G.; Gottfries, C. G.; Jostell, K.-G.; Karlsson, I.; Svennerholm, L. *Psychopharmacology* 1983, *80*, 279.

(47) Cutler, N. R.; Haxby, J.; Kay, A. D.; Narang, P. K.; Lesko, L. J.; Costa, J. L.; Ninos, M.; Linnoila, M.; Potter, W. Z.; Renfrew, J. W.; Moore, A. M. *Arch. Neurol.* 1985, *42*, [illegible].

(48) Arnsten, A. F. T.; Goldman-Rakic, P. S. *Science (Washington, D.C.)* 1985, *230*, 1273.



peutic interventions that alleviate memory impairments.

For the purposes of discussion, biological models of senile cognitive decline will be divided into three major neuropharmacological categories: biochemistry, electrophysiology, and behavior. The past generation of cognition activators was developed almost entirely through leads discovered during the course of behavioral testing. The present generation of agents represents a shift to better defined mechanisms of action wherein leads are identified through combined evaluation in all three areas of neuropharmacology.

For example, consider the cholinergic hypothesis, which has been proposed to explain the pathology and symptoms of geriatric memory dysfunction.[49] An impressive amount of research has been directed by this rationale in the 1980s.[50] If indeed the cholinergic deficits observed in PDD cause the cognitive decline observed, then, in principle, symptomatic treatment should be possible with several types of cholinergic agents. (However, activation of just one neurotransmitter system may not be enough to overcome the symptoms associated with PDD.)

Mechanistic questions are best addressed at an early stage through biochemical studies because of high testing throughput and minimal complicating pharmacokinetic and metabolic factors. In a cholinergic approach, these investigations might include a variety of assays: muscarinic receptor binding, high-affinity choline uptake, acetylcholine release, choline acetyltransferase activity, acetylcholinesterase activity, phosphatidylinositol turnover.

These assays can provide primary mechanistic models of senile cognitive decline. Alone, their value is limited, but in tandem with electrophysiology and behavioral testing, biochemical studies serve to provide rapid, well-defined input regarding potential activity, thus directing more time consuming efforts efficiently. Examples of agents that may enhance muscarinic cholinergic neurotransmission by a defined biochemical mechanism are illustrated in Table IV.

Brain electrical activity can be studied with standard electroencephalographic equipment. Coupled with behavioral studies, certain electrical changes have been correlated with attentional demands, emotional processes, and cognitive processes,[51] as outlined in Table V. Through this correlation, electrophysiology functions as a secondary mechanistic model for senile cognitive decline, and can serve in addition to provide information on duration of action, time course, time of peak effect, and potential toxicity.

Behavioral studies represent the penultimate endpoint in the development of drugs to treat senile cognitive decline, and a number of behavioral models exist at the present time (see Table VI). The discussion that follows summarizes and updates some recent reviews on this subject.[52]

Table VI. Behavioral Models

central nervous system (CNS) lesions
electrical (e.g., electroconvulsive shock (ECS))
genetic deficiencies
hypoxia/anoxia and ischemia
aged vs. young animals
drug-induced deficits

CNS Lesions. Studies of biochemical and histopathological changes in PDD patients, particularly in the cholinergic system, have suggested new approaches to developing animal models of senile cognitive decline. Ventral pallidal lesions produced by ibotenic acid do not alter rat performance on psychomotor tasks or affect sensitivity to shock.[53] However, severe deficits in retention of a passive avoidance response are found in these lesioned animals. Similar deficits are found in rats lesioned bilaterally in the ventral pallidum with use of another excitatory neurotoxin, kainic acid. Ethylcholine mustard aziridinium ion (AF64A), a neurotoxic choline analogue, produces long-lasting hypofunction of central cholinergic systems in mice and reduces presynaptic cholinergic markers in the rat hippocampus without affecting postsynaptic muscarinic receptor binding.[54] AF64A lesions may eventually provide an animal model of PDD, but behavioral evidence is preliminary. The use of cholinergic false precursors has also been suggested as a method for producing animals with cholinergic hypofunction.[55]

(49) Bartus, R. T.; Dean, R. L., III; Beer, B.; Lippa, A. S. *Science (Washington, D.C.)* 1982, *217*, 408. Mash, D. C.; Flynn, D. D.; Potter, L. T. *Science (Washington, D.C.)* 1985, *228*, 1115. Wurtman, R. J.; Blusztajn, J. K.; Maire, J.-C. *Neurochem. Int.* 1985, *7*, 369. Sitaram, N. *Drug Dev. Res.* 1984, *4*, 481. For another hypothesis, see, for example: Lynch, G.; Baudry, M. *Science (Washington, D.C.)* 1984, *224*, 1057.

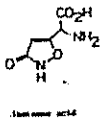
(50) *Alzheimer's Disease. Report of the Secretary's Task Force on Alzheimer's Disease*; U.S. Department of Health and Human Services, September, 1984, DHHS Publication No. (ADM) 84-1323.

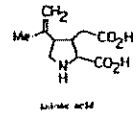
(51) Bay, W. J.; Cole, H. W. *Science (Washington, D.C.)* 1985, *228*, 750. Duffy, F. H.; Albert, M. S.; McAnulty, G. *Ann. Neurol.* 1984, *16*, 439. Duffy, F. H.; Albert, M. S.; McAnulty, G.; Garvey, A. J. *Ann. Neurol.* 1984, *16*, 430. Bennett, T. L.; Hebert, P. N.; Moss, D. E. *Behav. Biol.* 1973, *8*, 196. Green, J. D.; Arduini, A. *J. Neurophysiol.* 1954, *17*, 533. Dustman, R. E.; LaMarche, J. A.; Cohn, N. B.; Shearer, D. E.; Talone, J. M. *Neurobiol. Aging* 1985, *6*, 193.

(52) Olton, D. S.; Gamzu, E.; Corkin, S., Eds. *Ann. N.Y. Acad. Sci.* 1985, *444* (for a summary, see: Schwam, E.; Gamzu, E.; Vincent, G. *Neurobiol. Aging* 1984, *5*, 243). Hershenson, F. M.; Marriott, J. G. *Annu. Rep. Med. Chem.* 1984, *19*, 31. Hershenson, F. M.; Marriott, J. G.; Moos, W. H. *Annu. Rep. Med. Chem.*, in press.

(53) For an example of recent work with ibotenic acid, see: Dunnett, S. B. *Psychopharmacology* 1985, *87*, 357.

(54) Vickroy, T. W.; Watson, M.; Leventer, S. M.; Roeske, W. R.; Hanin, I.; Yamamura, H. I. *J. Pharmacol. Exp. Ther.* 1985, *235*, 577. Fisher, A.; Mantione, C. R.; Abraham, D. J.; Hanin, I. *J. Pharmacol. Exp. Ther.* 1982, *222*, 140. Stwertka, S. A.; Olson, G. L. *Life Sci.* 1986, *38*, 1105. An aziridinium analogue of oxotremorine (BM 130A) has also been studied (Russell, R. W.; Jackson, K. D.; Booth, R. A.; Jenden, D. J. *Psychopharmacology* 1986, *88*, 24).





ECS Models. Electroconvulsive shock has been used to produce severe retrograde amnesia, an effect well-documented at the clinical level and extensively studied in animals. The effects of agents on impaired memory in depressed patients undergoing ECS therapy are under study.[56] Since many cognition activators were discovered and developed on the basis of activity against ECS-induced amnesia, these studies will test the predictive value of this preclinical model.

Genetic Models. Natural deficits can be observed in certain genetic strains. For example, hippocampally deficient mice[57] are impaired in acquisition and retention with regard to finding a hidden platform in a water "maze".

Hypoxia Models. Low levels of oxygen induce electrophysiological changes and disrupt learning and memory. Even certain biochemical effects caused by hypoxia parallel those seen in aging. For example, treatment of spontaneously hypertensive rats with hypertonic saline causes behavioral deficits, and a morphology similar to that observed in multiinfarct dementia.

Aged Models. Old animals are used extensively as models of age-related cognitive disorders. Regional changes in brain glucose metabolism reflect cognitive impairments in aged rats.[58] Old mice are impaired on passive avoidance compared to young mice. In contrast with clinical data, dietary phosphatidylcholine enhances performance of old mice in shuttlebox avoidance. Aged rats perform at chance levels after 15 training trials using a 12-arm radial maze, whereas young rats master the task. Positive correlations in aged rats have been found between maze performance and hippocampal choline acetyltransferase activity. Aged monkeys have been employed in studies of age-related memory impairments and drug effects upon memory. Drug trials in monkeys have demonstrated effects with cholinergic agents and neuropeptides similar (i.e., marginal efficacy) to those reported in human trials.

Drug-Induced Deficit Models. Anticholinergic-induced cognitive deficits have also been used as a model of age-related impairments, with agents tested for their ability to reverse the deficits. Systemically administered atropine increases running time and working memory errors in mice trained on a six-arm radial maze. In a water maze, atropine-treated rats are impaired with respect to finding a hidden escape platform. Similar deficits are found in rats with total hippocampalectomy. Atropine disrupts and physostigmine enhances acquisition of light/dark discrimination and tone/no-tone discrimination in rats. Anticholinergics are also effective in disrupting memory when injected directly into the brain. Conversely, cholinergic agents (e.g., arecoline, physostigmine, oxotremorine, muscarine) improve retention on an active avoidance task when administered intracerebroventricularly after training and prior to retention testing 1 week later. MCI-2016 [4-(o-benzylphenoxy)-*N*-methylbutylamine] reverses scopolamine-induced impairments of spontaneous alternation responding in rats similar to the effects of physostigmine, choline, and amphetamine.

Benzodiazepine-induced amnesia, which was first described as a result of clinical experience, has been used as an animal model of amnesia.[59]

Are the Models Valid? *An unequivocal answer to this question may not be possible until a truly efficacious drug is discovered, thus allowing a comparison of preclinical and clinical results.* However, given a variety of agents that show some preclinical activity, the following scenarios pertain. (1) Perhaps the models are valid, but greater preclinical efficacy is needed. In this case we should seek drugs with more robust preclinical effects. (2) Perhaps side effects, a short duration of action, or a narrow active dose range mask the efficacy of useful drugs. Here, agents with fewer side effects, longer duration, and wider active dose ranges are needed. (3) Perhaps patient populations have been inadequately selected for clinical evaluation. If this is true, then we must develop means of accurately diagnosing varied types of senile cognitive decline, for example, with imaging techniques. (4) Perhaps the clinical symptoms of senile cognitive decline cannot be treated with drugs. If this is true, then efforts might be focused on prevention of senile cognitive decline or on surgical intervention, for example, with brain tissue transplants.[60]

Future Directions. The cognition activators currently under development are a diverse group. Whether these agents prove effective remains to be seen. Future cognition activators should not only act via defined mechanisms but should also possess undisputed efficacy. Whether the next generation arises from a series of incremental advances or a significant breakthrough, a major new era in neurosciences will be ushered in.

(55) Newton, M. W.; Crosland, R. D.; Jenden, D. J. *J. Pharmacol. Exp. Ther.* **1986**, *235*, 167.
(56) Ernst, D. H.; Ebrahesen, M. M.; Makhawy, B. *Br. J. Psychiatry* **1986**, *147*, 720.
(57) Symmes, J. P.; Davis, R. E.; Marriott, J. G. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1984**, *43*, 924 (Abstr. 3741).
(58) Gage, F. H.; Kelly, P. A. T.; Björklund, A. *J. Neurosci.* **1984**, *4*, 2856.
(59) Ganzen, E. R. *J. Clin. Psychiatry*, in press. Garattini, E.; Perrone, L.; Sebelix, R. *Bull. Psychonomic Soc.* **1979**, *13*, 253.
(60) Fine, A.; Dunnett, S. B.; Björklund, A.; Iversen, S. D. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 5227. Björklund, A.; Gage, F. H. *Ann. N.Y. Acad. Sci.* **1985**, *457*, 53. *Medical World News* **1985**, *26*, 8. Gage, F. H.; Björklund, A.; Stenevi, U.; Dunnett, S. B.; Kelly, P. A. T. *Science (Washington, D.C.)* **1984**, *225*, 533.